REMARKS

The claims have been amended to conform to US patent practice. Claims 6, 68 and 69 have been cancelled. The specification has been amended to add section headings.

Applicants contend that such amendment adds no new matter and finds support in the specification. Attached hereto, please find pages captioned "Version with markings to show changes made."

Conclusion

Applicants submit that the instant application is in condition for allowance. Accordingly, early examination and a Notice of Allowance are respectfully requested for Claims 1-5, 7-67 and 70-72. If the Examiner is of the opinion that the instant application is in condition for other than allowance, the Examiner is requested to contact the Applicants' Attorney at the telephone number given below so that additional changes may be discussed.

Respectfully submitted,

By _____

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Version with markings to show changes made.

IN THE SPECIFICATION:

Please delete the title and insert the following in its stead:
--NUCLEIC ACIDS WHICH CODE FOR THE ENZYME ACTIVITIES OF THE SPINOSYN BIOSYNTHESIS--. A new Abstract page is included herewith.

After the title please insert the following:

--FIELD OF THE INVENTION --

Please insert the following on page 1, line 7:

--BACKGROUND OF THE INVENTION--

Please insert the following on page 3, line 9:

--DETAILED DESCRIPTION OF THE INVENTION--

IN THE CLAIMS:

Please cancel claims 6, 68 and 69 without prejudice.

Please amend the claims as follows:

1. (Amended) A n[N]ucleic acid[, which] compris[es]ing at least one region coding for an enzyme activity [which is] involved in the biosynthesis of spinosyn[s].

2. (Amended) The n[N]ucleic acid [according to]of Claim 1[, characterized in that it is]comprising a single-stranded or double-stranded DNA or RNA.

3. (Amended) The n[N]ucleic acid [according to]of Claim 2[, characterized in that it is]comprising a DNA fragment.

4. (Amended) The n[N]ucleic acid [according to]of Claim 3[, characterized in that it] compris[es]ing all regions coding for enzyme activities which are involved in biosynthesis of spinosyn[s].

5. (Amended) The n[N]ucleic acid [according to any] of Claim[s] 1 [to 4, characterized in that the]wherein said enzyme activit[ies are of]y is selected from the group consisting of polyketide synthase[s], methyltransferase[s], glycosyltransferase[s], epimerase[s], aminotransferase[s], dimethyltransferase[s], reductase[s], dehydratase[s] and[/or] cyclization enzyme[s].

7. (Amended) A n[N]ucleic acid [according to Claim 1]comprised of at least one region which codes for an enzyme activity involved in the biosynthesis of spinosyn, comprising at least one sequence selected from

(a) [the sequences according to] SEQ ID NO[S:] 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 52 or 54,

(b) a part sequence[s of] which is at least 14 base pairs in length of the sequences defined [under] in (a),

(c) a sequence[s] which hybridizes to [the]a sequence[s] defined [under] in (a),

(d) a sequence[s] which [are]is at least 70% identical to [the]a sequence[s] defined [under]in (a),

(e) a sequence[s] which [are] is complementary to [the] a sequence[s] defined [under]in (a), and

(g) a sequence[s] which, due to the degeneracy of the genetic code, codes for the same amino acid sequence as [the]a sequence[s] defined [under]in (a) to (d).

8. (Amended) The n[N]ucleic acid [according to]of Claim 7[, characterized in that it] compris[es the]ing a sequence according to SEQ ID NOS: 1 to 6.

9. (Amended) The n[N]ucleic acid [according to]of Claim 7[, characterized in that it] compris[es the]ing a sequence according to SEQ ID NO: 4.

10. (Amended) The n[N]ucleic acid [according to]of Claim 7[, characterized in that it] compris[es the]ing a sequence according to SEQ ID NOS: 5 and 6.

11. (Amended) The n[N]ucleic acid [according to]of Claim 7[, characterized in that it] compris[es]ing at least one sequence according to SEQ ID NOS: 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 or 39.

12. (Amended) The n[N]ucleic acid [according to]of Claim 7[, characterized in that it] compris[es]ing at least one sequence according to SEQ ID NOS: 41, 43, 45, 47 or 49.

13. (Amended) A r[R]egulatory region, which controls transcription of a nucleic acid comprised of at least one region which codes for an enzyme activity involved in the biosynthesis of spinosyn [according to any of Claims 1 to 7] in *Saccharopolyspora spinosa*.

14. (Amended) A DNA construct comprising a nucleic acid [according to any of Claims 1 to 12] comprised of at least one region which codes for an enzyme activity involved in the biosynthesis of spinosyn and at least one heterologous promoter.

15. (Amended) A v[V]ector comprising at least one nucleic acid [according to any of Claims 1 to 12] comprised of at least one region which codes for an enzyme activity involved in the biosynthesis of spinosyn, a regulatory region [according to Claim 13]which controls transcription of said nucleic acid or a

DNA construct comprising said nucleic acid and at least one heterologous promoter[according to Claim 14].

16. (Amended) The v[V]ector [according to]of Claim 15, [characterized in that]wherein the nucleic acid is functionally linked to regulatory sequences which ensure expression of the coding regions of the nucleic acid in prokaryotic or eukaryotic cells.

17. (Amended) The v[V]ector [according to either] of Claim[s] 15 [and 16, characterized in that it is]comprised of a BAC vector, PAC vector or a vector functionally equivalent to BAC or PAC vectors.

18. (Amended) The v[V]ector [according to]of Claim 17[, characterized in that it is a vector corresponding to] selected from the BAC clones having the deposition numbers DSM 13010, DSM 13011 [or] and DSM 13012.

19. (Amended) The v[V]ector [according to any]of Claim[s] 15 [to 18, characterized in that it is]comprising a shuttle vector which can be transferred [both] to prokaryotes and to eukaryotes.

20. (Amended) The v[V]ector [according to any] of Claim[s] 15 [to 19, characterized in that it is]comprising a shuttle vector which can be transferred [both] to Gram-negative bacteria, [and] Gram-positive bacteria and [to] Archea.

21. (Amended) The v[V]ector [according to any] of Claim[s] 15 [to 19, characterized in that it is]comprising a shuttle vector which can be transferred [both] to *Escherichia coli* and to actinomycetes.

22. (Amended) The v[V]ector [according to]of Claim 21[, characterized in that it is]comprising a shuttle vector which can be transferred [both] to *Escherichia coli* and to *Streptomyces*.

23. (Amended) The v[V]ector [according to any] of Claim[s] 15 [to 22, characterized in that it]which can be replicated autonomously in a prokaryote.

24. (Amended) The v[V]ector [according to any] of Claim[s] 15 [to 22, characterized in that it]which can be integrated into the genome of a prokaryote [under involvement of]via the phage ΦC31 integration mechanism, the pSAM2 integration mechanism or the mini-circle integration mechanism.

25. (Amended) The v[V]ector [according to any] of Claim[s] 15 [to 22, characterized in that it]which can be integrated into the genome of a prokaryote by RecA-mediated recombination.

26. (Amended) The v[V]ector [according to any] of Claim[s] 15 [to 22, characterized in that it]which can be integrated into the genome of a prokaryote by RecE- and RecT-mediated recombination.

27. (Amended) A h[H]ost cell comprising a nucleic acid [according to any of Claims 1 to 12] comprised of at least one region which codes for an enzyme activity involved in the biosynthesis of spinosyn, a regulatory region [according to Claim 13]which controls transcription of said nucleic acid, a DNA construct [according to Claim 14]comprising said nucleic acid and at least one heterologous promoter or [at least] a vector [according to any of Claims 15 to 26]comprising said nucleic acid, said regulatory region or said DNA contstruct.

28. (Amended) The h[H]ost cell [according to]of Claim 27[, characterized in that it is]comprising a prokaryotic or eukaryotic cell.

29. (Amended) The h[H]ost cell [according to]of Claim 28, [characterized in that]wherein the prokaryotic cell belongs to the group of actinomycetes[, preferably the group of streptomycetes].

30. (Amended) The h[H]ost cell [according to]of Claim 28, [characterized in that]wherein the eukaryotic cell is a plant cell.

31. (Amended) A p[P]olypeptide [which is] encoded by a nucleic acid [according to any of Claims 1 to 7] comprised of at least one region which codes for an enzyme activity involved in the biosynthesis of spinosyn.

32. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having a methyltransferase activity.

33. (Amended) The p[P]olypeptide [according to]of Claim 32[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NOS: 8, 12, 14, 18 or 20, or a part sequence thereof.

34. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having a glycosyltransferase activity.

35. (Amended) The p[P]olypeptide [according to]of Claim 34[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NOS: 10 or 30, or a part sequence thereof.

36. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having the activity of a C-C linking enzyme which carries out cyclization reactions.

37. (Amended) The p[P]olypeptide [according to]of Claim 36[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NO: 16 or a part sequence thereof.

38. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having the activity of an enzyme which is involved in cyclization reactions.

39. (Amended) The p[P]olypeptide [according to]of Claim 38[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NO: 22 or a part sequence thereof.

40. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having a 2,3-reductase activity.

41. (Amended) The p[P]olypeptide [according to]of Claim 40[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NO: 24 or a part sequence thereof.

42. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having a 2,3-dehydratase activity.

43. (Amended) The p[P]olypeptide [according to]of Claim 42[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NO: 26 or a part sequence thereof.

44. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having a thioesterase activity.

45. (Amended) The p[P]olypeptide [according to]of Claim 44[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NO: 28 or a part sequence thereof.

46. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having a 3,4-dehydratase activity.

47. (Amended) The p[P]olypeptide [according to]of Claim 46[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NO: 32 or a part sequence thereof.

48. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having a 4-aminotransferase activity.

49. (Amended) The p[P]olypeptide [according to]of Claim 48[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NO: 34 or a part sequence thereof.

50. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having an N-dimethyltransferase activity.

51. (Amended) The p[P]olypeptide [according to]of Claim 50[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NO: 36 or a part sequence thereof.

52. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having a 3,4-reductase activity.

53. (Amended) The p[P]olypeptide [according to]of Claim 52[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NO: 38 or a part sequence thereof.

54. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having a transcription regulator activity.

55. (Amended) The p[P]olypeptide [according to]of Claim 54[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NO: 40 or a part sequence thereof.

56. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having a polyketide synthase activity.

57. (Amended) The p[P]olypeptide [according to]of Claim 56[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NOS: 42, 44, 46, 48 or 50, or a part sequence thereof.

58. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having a glucose dehydratase activity.

59. (Amended) The p[P]olypeptide [according to]of Claim 58[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NO: 53.

60. (Amended) The p[P]olypeptide [according to]of Claim 31[, characterized in that it has] having a 3,5-epimerase activity.

61. (Amended) The p[P]olypeptide [according to]of Claim 60[, characterized in that it has] comprising the amino acid sequence according to SEQ ID NO: 55.

62. (Amended) An e[E]nzyme[s which are] involved in a cyclization reaction[s], [characterized in that they] compris[e]ing the amino acid sequence according to SEQ ID NO: 15 or 22 or a part sequence thereof which is [still] able to carry out at least part of [the]said reaction or [in that they are]has an amino acid sequence at least 50% identical thereto [at the amino acid level].

63. (Amended) An a[A]ntibody, which reacts specifically with a polypeptide [according to any] of Claim[s] 31 [to 62].

64. (Amended) A m[M]ethod for preparing a nucleic acid [according to any of Claims 1 to 7] comprised of at least one region which codes for an enzyme activity involved in the biosynthesis of spinosyn, said method comprising [the following steps]:

[(a) complete chemical synthesis in a manner known per se] synthesizing the complete nucleic acid by chemical methods or

[(b) chemical synthesis of]synthesizing oligonucleotides by chemical methods,
labelling [of the]said oligonucleotides,
hybridizing [the]said oligonucleotides to DNA of a genomic or cDNA library which has been prepared [by starting] from genomic DNA or mRNA from *S. spinosa*,
selecting positive clones and
isolating the hybridizing DNA from positive clones or

[(c) chemical synthesis of]synthesizing oligonucleotides by chemical methods and
amplif[ication of]ying the target DNA by [means of] PCR.

65. Method for preparing a polypeptide encoded by a nucleic acid comprised of at least one region which codes for an enzyme activity involved in the biosynthesis of spinosyn [according to any of Claims 31 to 62], said method comprising [the following steps]:

[(a)] culturing a host cell [according to any of Claims 27 to 30]comprising said nucleic acid, a regulatory region which controls transcription of said nucleic acid, a DNA construct comprising said nucleic acid and at least one heterologous promoter or a vector comprising said nucleic acid, said regulatory region or said DNA construct under conditions which ensure expression of [the]said nucleic acid [according to any of Claims 1 to 7], or

[(a1)] expressing [a]said nucleic acid [according to any of Claims 1 to 7] in an *in vitro* system, and

[(b)] obtaining the polypeptide from the cell, the culture medium or the *in vitro* system.

66. (Amended) A m[M]ethod for preparing spinosyn, spinosyn precursors or spinosyn derivatives, comprising [the following steps]:

[(a)] culturing a host cell [according to any of Claims 27 to 30] comprising a nucleic acid comprised of at least one region which codes for an enzyme activity involved in the biosynthesis of spinosyn, a regulatory region which controls transcription of said nucleic acid, a DNA construct comprising said nucleic acid and at least one heterologous promoter or a vector comprising said nucleic acid, said regulatory region or said DNA construct under conditions which ensure expression of [the]said nucleic acid [according to any of Claims 1 to 7], and

[(b)] obtaining the spinosyn, spinosyn precursor or spinosyn derivative from the cell or the culture medium.

67. (Amended) A m[M]ethod for preparing spinosyn derivatives, including spinosyn precursors, comprising [the following steps]:

[(a)] exchanging at least one module-encoding nucleic acid sequence according to Claim 7 for at least one other module-encoding nucleic acid sequence according to Claim 7, or

[(b)] exchanging at least one module-encoding nucleic acid sequence according to Claim 7 for at least one other module-encoding nucleic acid sequence from *S. spinosa*, or

[(c)] exchanging at least one module-encoding nucleic acid sequence according to Claim 7 for at least one other module-encoding nucleic acid sequence from an organism other than *S. spinosa*, or

[(d)] exchanging at least one domain-encoding nucleic acid sequence according to Claim 7 for at least one other domain-encoding nucleic acid sequence according to Claim 7, or

[(e)] exchanging at least one domain-encoding nucleic acid sequence according to Claim 7 for at least one other domain-encoding nucleic acid sequence from *S. spinosa*, or

[(f)] exchanging at least one domain-encoding nucleic acid sequence according to Claim 7 for at least one other domain-encoding nucleic acid sequence from an organism other than *S. spinosa*, or

[(g)] exchanging a first acyltransferase-encoding nucleic acid sequence according to Claim 7 for a second acyltransferase-encoding nucleic acid sequence according to Claim 7, wherein the second acyltransferase has a substrate specificity different from that of the first acyltransferase, or

[(h)] exchanging a first acyltransferase-encoding nucleic acid sequence according to Claim 7 for a second acyltransferase-encoding nucleic acid sequence from *S. spinosa*, wherein the second acyltransferase has a substrate specificity different from that of the first acyltransferase, or

[(i)] exchanging a first acyltransferase-encoding nucleic acid sequence according to Claim 7 for a second acyltransferase-encoding nucleic acid sequence from an organism other than *S. spinosa*, wherein the second acyltransferase has a substrate specificity different from that of the first acyltransferase, or

[(j)] deleting at least one domain-encoding nucleic acid sequence according to Claim 7, or

[(k)] integrating at least one domain-encoding nucleic acid sequence according to Claim 7 into a module-encoding nucleic acid sequence according to Claim 7, or

[(l)] mutating at least one domain-encoding nucleic acid sequence according to Claim 7,

and expressing the recombinant nucleic acid sequence in a host cell under conditions which allow synthesis of a spinosyn derivative or a spinosyn precursor.

70. (Amended) A m[M]ethod for attaching a forosamine sugar residue to the spinosyn aglycone or to the spinosyn 17-pseudoaglycone or to a polyketide aglycone, comprising [the following steps]:

[(a)] transferring a nucleic acid according to SEQ ID NOS: 23, 25, 29, 31, 33, 35 and 37 into a host cell which can produce the spinosyn aglycone or the spinosyn 17-pseudoaglycone or the polyketide aglycone, or

[(a1)] transferring a nucleic acid according to SEQ ID NOS: 23, 25, 29, 31, 33, 35 and 37 into a host cell which cannot produce the spinosyn aglycone or the spinosyn 17-pseudoaglycone or the polyketide aglycone and adding the spinosyn aglycone or the spinosyn 17-pseudoaglycone or the polyketide aglycone to the culture medium, and

[(b)] culturing the host cell under conditions which lead to an active cell metabolism.

71. (Amended) A m[M]ethod for attaching a trimethylrhamnose sugar residue to the spinosyn aglycone or the spinosyn 9-pseudoaglycone or to a polyketide aglycone, comprising [the following steps]:

[(a)] transferring a nucleic acid according to SEQ ID NO: 7, 9, 11, 13, 17 and/or 19 into a host cell which can produce the spinosyn aglycone or the spinosyn 9-pseudoaglycone or the polyketide aglycone, or

[(a1)] transferring a nucleic acid according to SEQ ID NO: 7, 9, 11, 13, 17 and/or 19 into a host cell which cannot produce the spinosyn aglycone or the spinosyn 9-pseudoaglycone or the polyketide aglycone and adding the spinosyn aglycone or the spinosyn 9-pseudoaglycone or the polyketide aglycone to the culture medium, and

[(b)] culturing the host cell under conditions which lead to an active cell metabolism.

72. (Amended) The m[M]ethod [according to]of Claim 71, [characterized in that in step (a)]wherein nucleic acids according to SEQ ID NOS: 9, 11, 13 and 17 are transferred.

NUCLEIC ACIDS WHICH CODE FOR THE ENZYME ACTIVITIES OF THE SPINOSYN BIOSYNTHESIS

ABSTRACT OF THE DISCLOSURE

The present invention relates to nucleic acid coding for enzyme activities of spinosyn biosynthesis and to the relevant enzymes per se. Furthermore, the invention relates to methods for preparing spinsoyn derivatives and spinosyn precursors.